

STEM ANATOMY OF DIOON SPINULOSUM

CONTRIBUTIONS FROM THE HULL BOTANICAL LABORATORY 270

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(WITH PLATES XV-XVII AND FOUR FIGURES)

Introduction

Investigations dealing with the minute anatomical structure of the adult cycad stem have become very numerous and more or less thorough for all of the genera and many of the species, with the exception of the great Mexican representative, *Dioon spinulosum*. This unique and interesting species was first but inadequately described by EICHLER (6) in 1883, and by DYER (5) in 1885. The first extensive account of its general field characters, size, external structure, and distribution was by CHAMBERLAIN (1) in 1909. A later article (2) by the same author gives a full and careful description of the macroscopic structure of adult stems of *Dioon spinulosum*, *D. edule*, *Ceratozamia mexicana*, and *Zamia floridana*, particular attention being given to *D. spinulosum*. Special study is made of the growth rings, reported here for the first time in cycads, and of the medullary bundles which constitute the vascular system of the cones, and which are called cone domes, because of the domelike arrangement of these strands with the peduncle of the cone at their apex. The histological characters of the trunk, its growth rings, the thick-walled fibers of the phloem, and the structure of the xylem elements the author considers remarkably similar to the corresponding structures of *Cycadeoidea*.

The embryo and seedling of *D. spinulosum* have been studied recently by Sister HELEN ANGELA (4), and found in the arrangement and orientation of the vascular strands in the cotyledons, hypocotyl, stem, and leaves to differ in no marked degree from the usual cycad arrangement. Features particularly worthy of note in connection with the girdling habit, as this investigator has traced it from macerated seedling stems, is that each leaf is supplied with five strands arising from cauline bundles situated in different parts

of the stem, and further, that these girdling strands are horizontal from the beginning and continue so throughout their whole extent.

The investigation here described was undertaken with a view to supplementing CHAMBERLAIN'S general account of the histological structure of the adult stem of *D. spinulosum*, especially by a careful study of the broad foliar rays or leaf gaps with their included traces, a phase of cycadean anatomy only slightly touched upon by earlier investigators. As the work has progressed, its scope has been extended to include the general course and organization of the foliar strands in the cortical part of both adult and seedling stems.

Material and methods

The adult wood and abundant material of two- and three-year-old seedlings, which furnish the basis for this study, were secured by CHAMBERLAIN from the Hacienda de Joliet near Tierra Blanca, Mexico. The ten-year-old seedlings were from seeds procured in the same locality but germinated and grown in the botanical greenhouse of the University of Chicago.

Preparation of all material of the adult stem for sectioning was in the main as follows. Narrow, wedge-shaped sections extending radially from pith to cortex were cut from both the upper and lower portions of a trunk 18 ft. in height, care being taken that each included two or more of the large medullary rays. These sections were then cut into blocks about 1 cm. square, some slightly larger, and kept in series.

The various stages involved in the preparation of these blocks for sectioning, namely, demineralization of the woody tissues through the use of hydrofluoric acid, followed by a thorough washing of the material in running water to free it from all traces of the reagent, transference to various grades of alcohol and xylol, and finally imbedding in paraffin, have been discussed in a previous paper (8). Special care had to be taken in imbedding, the best results being obtained when the blocks were carried through the process of infiltration with paraffin from 48 hours to 3 days. After this they could easily be cut with a sliding microtome, and a complete series obtained by removing each section, as cut, from the knife and placing it directly upon the slide. Staining was with

safranin and gentian violet, or safranin and "licht grün," the latter combination proving the more satisfactory.

The greater part of the study of the girdling habit in the seedlings was made from cleared material. Entire sections comprising stem cylinder and cortex, in blocks 0.5×1 inch, were cleared so perfectly that it was possible, with the aid of a strong artificial light, to look through such a section and see the vascular strands clearly outlined in the cortex.

In the case of the two- and three-year-old seedlings, the method followed consisted in severing the long taproot from the stem just below the region of the cotyledonary plate and cutting off the long terminal leaves, leaving only the leaf bases and a small part of the petiole. The scale leaves were then carefully trimmed from two sides of the stem, and one clean longitudinal cut made through the entire stem from apex to base. After the transference of these half-sections to 50 per cent alcohol (each seedling being kept in a separate receptacle), the process was substantially the same as that for the paraffin method, that is, up to the pure xylol stage. At this point the material was subjected to vacuum treatment in order to free the tissues, as far as possible, of any air or gases they might contain. As a final clearing agent a mixture of xylol and carbon disulphide was used; the CS_2 , having a higher refractive index, rendered the material more transparent than pure xylol.

Adult stem

With the single exception of the Australian *Macrozamia Hopei*, *Dioon spinulosum* Dyer is the tallest of all cycads, ranging 10-30 ft. in height, with occasional specimens reaching 40 and even 50 ft. The particular specimen from which this study was made was about 18 ft. tall and possibly 100 years old. The width of the woody zone from pith to cortex averages 0.5-0.75 inch in the upper part of the trunk and 3.5-4 inches in the lower trunk.

STRUCTURE OF XYLEM.—The adult stele of *D. spinulosum* is endarch, and its compact woody cylinder consists chiefly of longitudinal tracheidal elements and radial parenchyma. From the pith to the cortical part of the stem the length of the tracheids averages as follows: scalariform metaxylem tracheids 4-4.2 mm.,

first pitted tracheids 5-6.5 mm., tracheids in the vicinity of the cambium 7-9.8 mm.

The protoxylem elements are of the reticulate and scalariform types, and in passing from the metaxylem to the first formed elements of the secondary wood all transitional stages occur in the reduction of the scalariform structure into imperfectly formed, multiseriate, bordered pits.

While the majority of the tracheids of the secondary xylem exhibit on their radial walls the multiseriate type of pitting so characteristic of this wood, many of the tracheidal elements, especially those constituting the secondarily formed wood in the upper trunk, have their radial walls covered with small bordered pits of a very irregular arrangement.

In the wood of the lower trunk tertiary spiral thickenings of the tracheid walls were observed occurring in the first few rings of growth and also in the older wood (fig. 1). These spirals are not common to all the tracheids, but are generally sporadic in their appearance and may be quite inconspicuous. In some cases, however, they are characterized by considerable prominence, and are so compact as to suggest a reticulate rather than a spiral formation.

In addition to the lignified elements of the wood there are narrow elongated cells with transverse walls, the longitudinal storage parenchyma. These cells, like those of the radially disposed

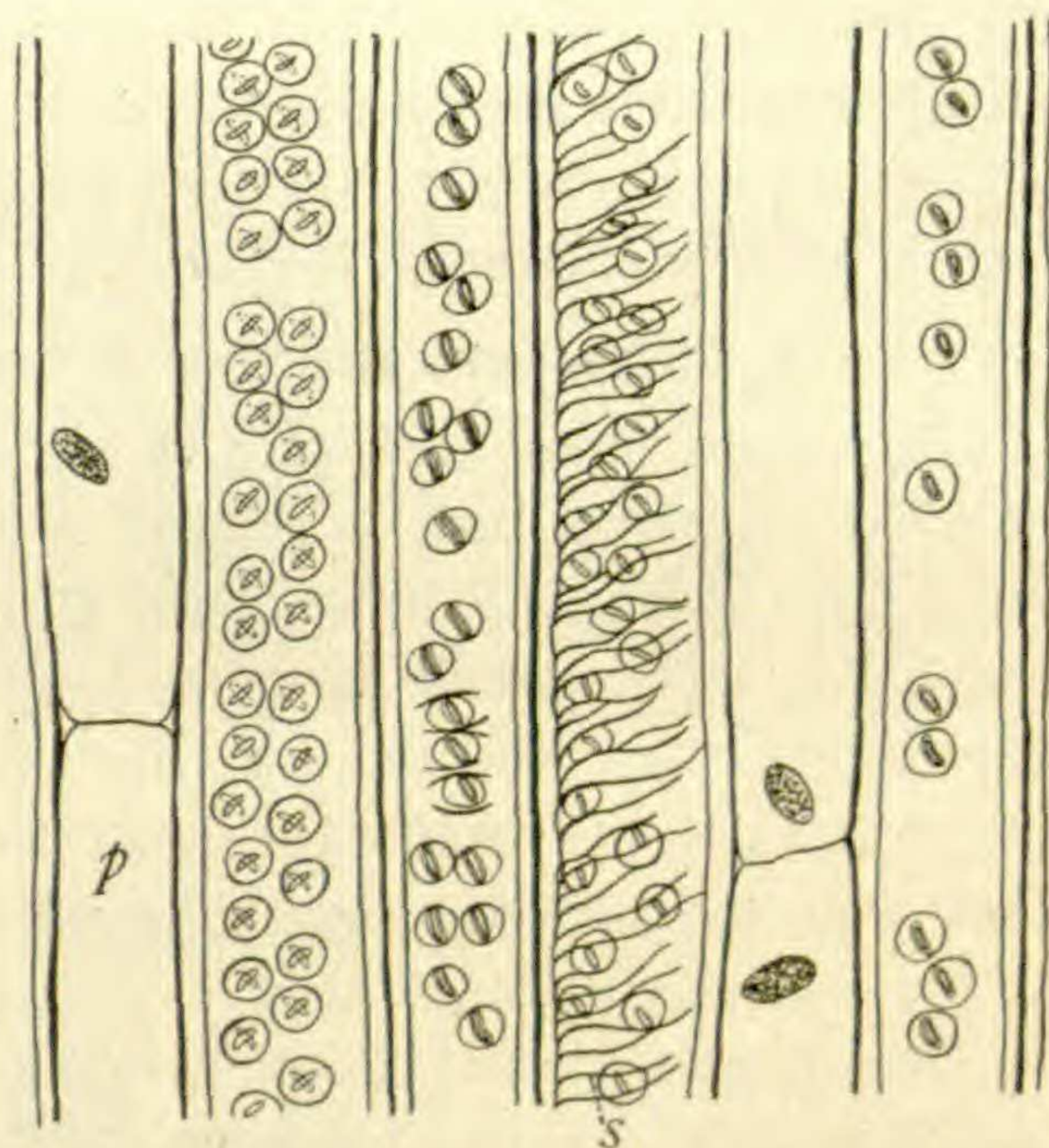


FIG. 1.—Radial longitudinal section of tracheids from lower portion of adult trunk: *s*, tertiary spiral thickenings of tracheid walls; *p*, wood parenchyma; $\times 225$.

bands of parenchyma, are usually well filled with starch and occasional calcium oxalate crystals.

MEDULLARY RAYS.—The medullary rays are of three types, namely, narrow uniseriate rays, a single layer of cells wide and several cells deep; multiseriate rays, two to several cells in width at their widest point and of variable longitudinal extent; and broad foliar rays or leaf gaps, which in tangential view resemble the aggregate ray of *Quercus*. The last are distributed at fairly equal intervals throughout the woody cylinder and always extend from the pith to the cortex. They are further characterized by the presence of at least one mucilage duct and one leaf trace bundle situated in the lower central part of the gap (fig. 6). A few isolated cases occur where two ducts and even two traces may be seen in a single foliar ray.

Course and structure of leaf trace in gap

The course of the leaf trace through the parenchymatous gaps or foliar rays from pith to phloem is approximately level, except for a slight downward curve of the strands due to their manner of formation. The bundle is endarch throughout its course in the gap and through the phloem, the xylem of the bundle usually uppermost and just beneath the duct, with no change in orientation until the bundle reaches the cortex, where it is continuous with one of the oblique cortical strands.

The manner of connection of this foliar trace with the primary and secondary wood of the main stele is one of the most striking and interesting features of the wood. Within a short distance of the pith the strands of the trace curve downward, the primary and secondary elements uniting with like elements of the main cylinder. On the interior vertical face of the wood at the point of union, and continuing up through the gap, always on the upper side of the trace (figs. 3-5) where the primary vessels of the trace would naturally appear, are peculiar tracheidal elements, curiously reticulated, in some cases forming continuous vessels, in other cases merely isolated patches of lignified tissue. These irregular fibrous elements are best illustrated in fig. 2, where a longitudinal section of the upper portion of the trace appears in a transverse section of the wood.

WORSDELL (12) also calls attention to the occurrence of peculiar irregular tracheids resembling "transfusion-tissue" on the interior vertical face of the wood and accompanying the bundles of the large medullary rays of *Macrozamia Fraseri*, and also found among

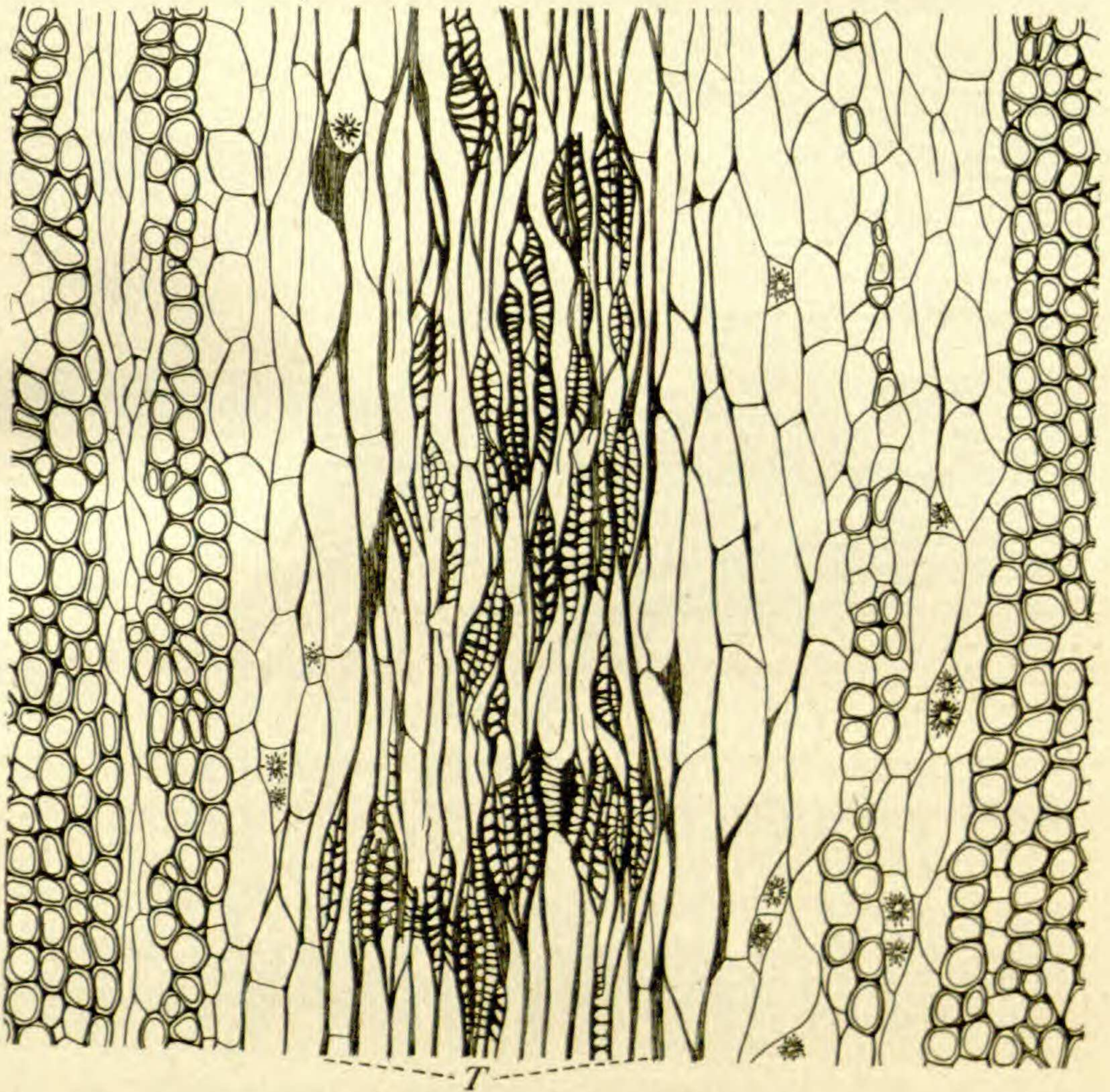


FIG. 2.—Transverse section of mature wood, showing foliar ray in center: *T*, longitudinal section through upper part of leaf trace; $\times 85$.

the parenchyma cells between successive vascular rings in *Encephalartos* (13). These, however, are of a pitted type rather than of the irregularly reticulated and scalariform types characteristic of *D. spinulosum*.

The mode of connection of this trace with the secondary fibro-vascular structures of the stem is as follows. Figs. 3, 4, and 5 are

radial longitudinal sections of the large medullary ray with its included foliar strand. A careful study of a series of such radial sections through the trace has made it apparent that this connection is by means of long, irregularly shaped scalariform tracheids which are the real tracheids constituting the trace, and not mere con-

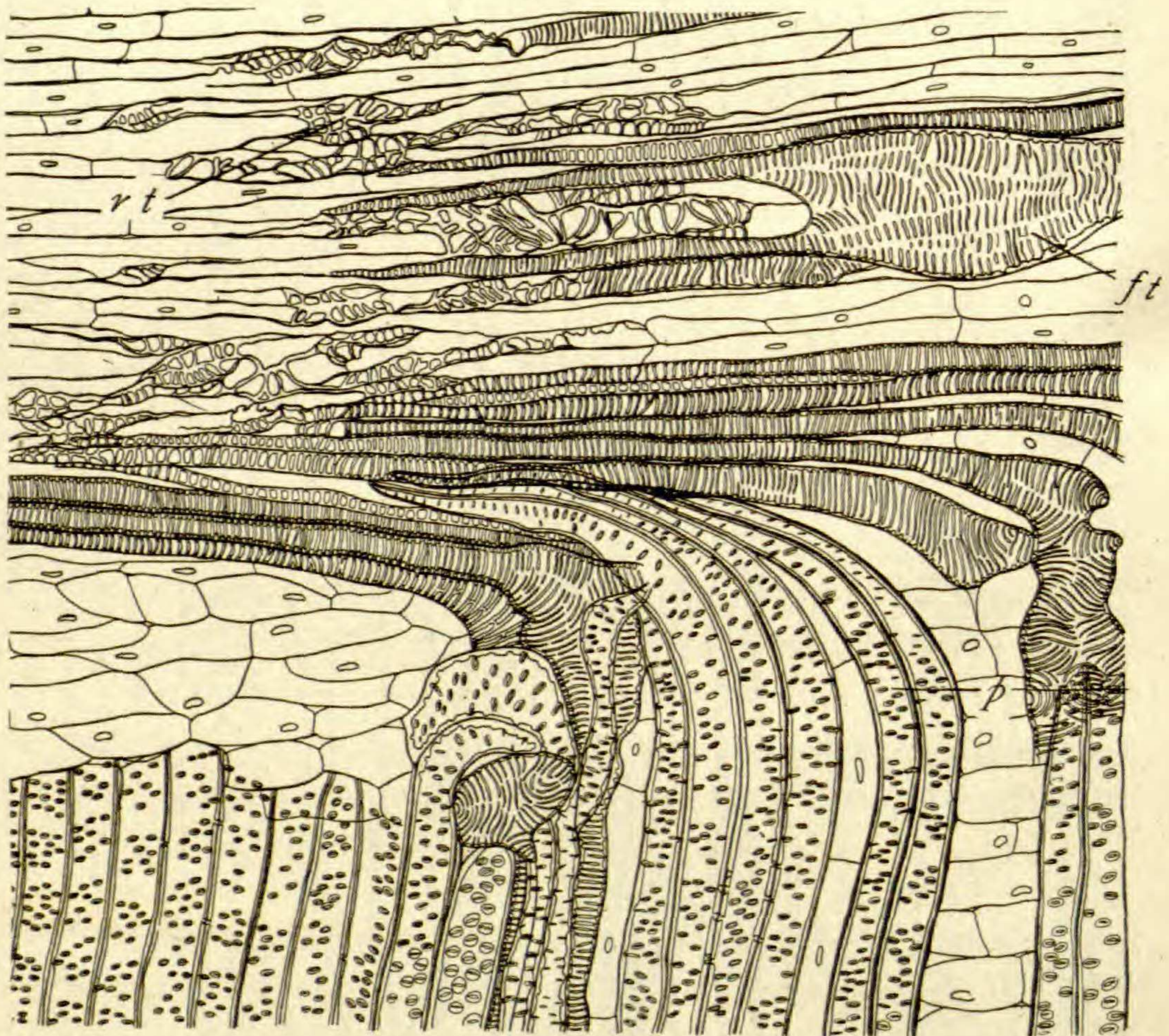


FIG. 3.—Radial longitudinal section of portion of large medullary ray with included foliar strand: *ft*, peculiar tracheidal formation apparently resulting from fusion of two or more tracheids; *rt*, irregularly reticulated elements; $\times 90$.

necting elements, as generally believed. These vessels may be entirely scalariform in structure, or may change gradually from a reticulate type at their tapering ends to decidedly scalariform throughout the greater part of their horizontal and vertical extent. As they extend horizontally through the gap they are arranged in order of formation, one vessel beneath the other (figs. 4, 5), the

lowest and last formed continuing a little farther in the direction of the cortex than the one preceding. In this way they constitute a perfectly continuous conducting system for solutions passing out to the cortical strands. These tracheids are especially numerous a short distance behind the pith, just beyond the primary xylem, but passing on through the gap they appear to become grouped (fig. 5), occurring at intervals with great masses of phloem and parenchyma separating the basal parts of each group. Whether this grouping is associated with a seasonal increase in the length of the trace, corresponding to the radial increase and growth rings of the main stele, has not been determined. In addition to the reticulate and scalariform elements constituting these traces, the regular pitted tracheids of the secondary xylem, usually at the extreme lateral borders of the gap, are often diverted to one side into a direction more or less parallel to that of the trace, as shown in figs. 3 and 5.

WORSDELL (12) has described in the case of *Macrozamia Fraseri* a somewhat similar connection between the fibrous strands in the large medullary rays and the fibrovascular elements of the main stele. He states that "a characteristic feature of the radial section of the wood is the large number of outbending strands of tracheids which, passing through the medullary rays, are continuous with the girdle leaf trace of the cortex." It is worthy of note, however, that these strands of outbending tracheids, or rather inbending in the sense that they are apparently diverted from the direction of the other secondary xylem elements toward that of the rays, are of the pitted type throughout their length, thus homologous with the pitted elements described in the preceding paragraph.

The peculiar down-curving growth of the scalariform tracheids constituting the foliar strands in the large medullary rays of *D. spinulosum* is another interesting illustration of the much discussed phenomenon of gliding growth. Vertically these conducting elements may extend merely to the lower borders of the gap and terminate in the irregular bulbous formations illustrated in fig. 4B, or they may become inserted for a considerable depth between the perpendicular fibrous elements of the main stele (fig. 5sc). In their horizontal extent these tracheids may be and probably are

the product of cambial activity, but in their vertical enlargement and elongation it seems probable that these lignified elements have simply been stretched out into their curious bending shapes by the growth of the adjoining living parenchyma tissue. The close scalariform markings on these vessels, in some cases approaching almost a pitted character, would indicate that this growth or elongation has taken place gradually, keeping pace with the longitudinal expansion of the gap.

It is also evident that the basal portions of many of these vessels have their origin in quite a different manner. The character of the pitting indicates that there has been a gradual lignification of the ordinary parenchyma cells (fig. 4 *B*), and a subsequent fusion by the breaking down and reabsorption of the partition walls. The formation of the peculiar curved and bulbous-like bases of many of these tracheids, where they come in contact with the perpendicular elements of the secondary wood, is shown in this way.

Course of leaf traces in cortex

The course of the fibrovascular bundles in the cortex, complicated by the well known habit of girdling, was first described by KARSTEN (7) in *Zamia muricata*, in 1856, later, in 1861, by METTENIUS (10), and in more recent articles quite fully by THIESSEN and Sister HELEN ANGELA in seedlings of *Ceratozamia* (3), *Dioon edule* (11), and *D. spinulosum* (4).

A brief statement of the girdling situation in the embryo and seedling of *Dioon edule*, as described by THIESSEN, is approximately as follows. For each leaf or scale leaf there are four distinct strands leaving the vascular cylinder. Two of these leave on the same side as the leaf for which they are destined, and pursue a direct course through the cortex to the central part of the petiole without branching; while the other two strands leave the cylinder approximately on the opposite side, describe a wide curve around it, and finally enter the dorsal part of the leaf petiole, where they branch repeatedly.

Sister HELEN ANGELA (4) has described a similar situation and arrangement of the cortical traces in the seedlings of *Dioon spinulosum*. Both authors agree that there are 4 or 5 strands leaving

the main cylinder for each leaf, each one of these strands describing a separate arc to the point where it enters a leaf base. Furthermore, all girdles are reported as being horizontal throughout their whole extent. It is obvious, therefore, that the phenomenon of girdling, as I have been able to trace it very distinctly and definitely in cleared specimens of two-, three-, and ten-year-old seedlings of *D. spinulosum*, differs in many respects from these earlier accounts. Thus for each leaf or scale leaf 7-9 strands, the number varying with the size of the sheathing leaf base, separate from the vascular cylinder. Two of these (fig. 8 *e*, *e*¹) leave the cylinder on the same side as the leaf for which they are destined and take an upward, oblique course for some distance, finally passing out more or less directly through the cortex into the ventral part of the petiole.

Two other traces (fig. 9 *a*, *a*¹) leave the main stele at closely approximated points on the side opposite the leaf for which they are destined and pursue an upward, rather oblique course for some distance. Then, curving one in either direction, they take a horizontal course, describing wide arcs through the cortex and sheathing leaf base, finally entering the dorsal or adaxial part of the petiole, where they undergo a complicated system of branching. The rest of the traces destined for this leaf (fig. 9 *b*, *b*¹, *c*, *c*¹, etc.) leave the main stele at intermediate points and assume, like traces (*a*, *a*¹), an upward, vertical direction, finally anastomosing with the two horizontal strands as they encircle the cortex. It is also noteworthy that each of these lateral oblique traces leaves the stem cylinder at a point slightly higher than the one preceding, so that the entire course of the two girdles is gradually and spirally ascending to the point where they enter the central part of the leaf base. Frequently a single bundle (fig. 8 *a*), separating from the vascular cylinder on the side opposite the leaf for which it is destined, may divide soon after leaving the central cylinder, the two horizontal branches swinging to right and left in wide curves through the cortex and the sheathing base of the leaf, and gradually anastomosing with the rest of the traces destined for that leaf. The character of the branching of these two main strands after entering the adaxial part of the petiole is so clearly illustrated in fig. 10 that any further discussion of this point is unnecessary.

At the very tip of the stem the traces of the youngest leaves ascend in an almost perpendicular direction about the region of the so-called potential vascular tissue to the point where they connect with the horizontal bundles. At this stage (fig. 10) all of the girdling strands lie in substantially the same plane, the pair associated with the youngest leaf describing slightly smaller arcs than those of the older leaves of the same crown. As internal radial growth and the appearance of new leaves crowd the older parts farther and farther away from their original terminal position, however, the lateral foliar traces become less vertical and more oblique. With this radial and longitudinal expansion of the stem is also associated a lengthening of the horizontal girdling strands, and consequently a widening of the intervals and the arcs between each lateral connecting trace.

These leaf traces are always endarch and collateral as they leave the stem cylinder, and also during their passage vertically and horizontally through the cortex to a point well up in the leaf base. They are so orientated that the xylem and phloem are directed toward the inside and the outside of the stem respectively. Transverse and longitudinal views (fig. 12) throw additional light on the organization of these cortical bundles.

COURSE OF LEAF TRACE IN ADULT STEM.—Due to the difficulties involved in following up strands of such size, it is impossible to determine with certainty whether the arrangement of the leaf traces in the adult stem of *D. spinulosum* is the same as that found characteristic of the seedlings. The problem becomes increasingly difficult as the plant reaches an age when the crown comprises numerous developing leaves. From longitudinal and transverse sections of the adult stem, however, it is evident that the same general relation between lateral oblique traces and a horizontal girdling strand is maintained, but it is probable that the girdling is only partial, that traces a and a^r (fig. 9) would have their origin at points more remote from each other in the adult stem. It is also probable that there is no appreciable increase in the number of traces associated with successive leaves, beyond the number described as supplying the leaves of the ten-year-old seedling (fig. 10).

Discussion

In his description of the girdling habit, METTENIUS (10) finds that "in the developmental stage the traces of the youngest leaves

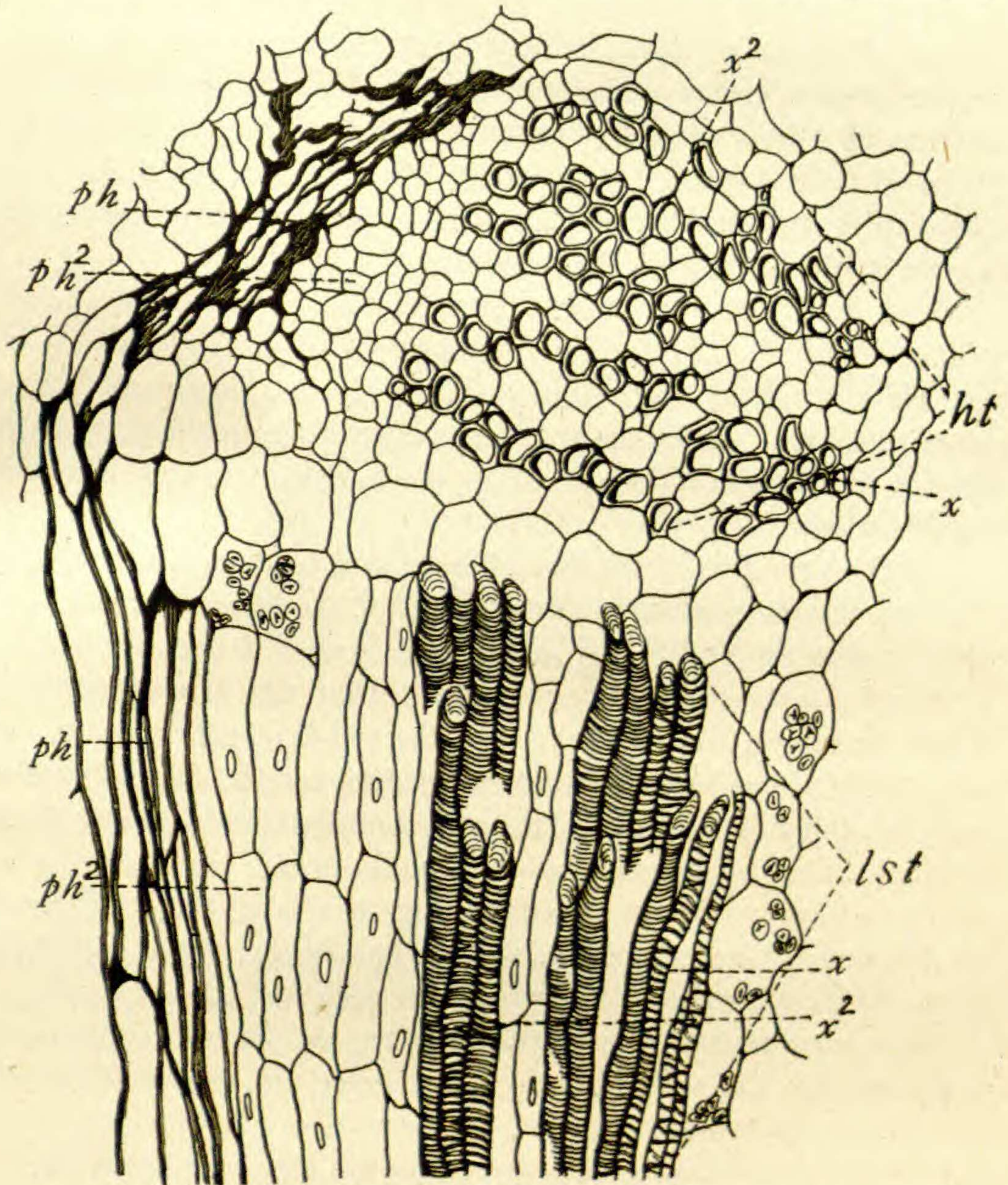


FIG. 12.—Detail of organization of cortical foliar strands of three-year-old plant: *ht*, horizontal strand in transverse section; *lst*, longitudinal section of vertical lateral trace near point of union with horizontal strand; *x*, primary sylem; *x*², secondary xylem; *ph*, primary phloem; *ph*², secondary phloem; $\times 120$.

lie in the region of the vegetative point, and at first ascend in an almost perpendicular direction, but during further growth assume

gradually a horizontal position, and with subsequent growth are lengthened and the expanse increased." MATTE (9) and Sister HELEN ANGELA (3), in connection with *Ceratozamia*, both describe a similar vertical position of traces in the early developmental stages, girdling becoming evident with the increase in diameter of the inclosed group of leaves and stem. In more recent investigations of *Dioon edule* by THIESSEN, and of *D. spinulosum* by Sister HELEN ANGELA (4), however, both authors maintain that the girdles are established very early, and that their horizontal course is laid down from the beginning.

The results of the present investigation indicate two possibilities therefore. Either the arrangement of cortical strands in the older seedlings and adult wood of *Dioon spinulosum* differs from that found in the embryo and very young seedlings of both species of *Dioon*, or the preceding statements need considerable modification.

At the very tips of the two-, three-, and ten-year-old seedlings (fig. 10) the perpendicular arrangement of the lateral strands and their connection with the horizontal girdles is unmistakable. It is reasonable to suppose, therefore, that the arrangement of foliar strands in the first leaves of the young seedling would be substantially the same as that characterizing the leaves of the older seedlings, save that (1) the very young strands having their origin in the cotyledonary plate would ascend vertically for a shorter distance before anastomosing to form the horizontal girdles, and (2) there would be likely to be a decrease in the number of strands leaving the vascular plate for each leaf base.

Another question of importance is the significant relationship suggested by the distribution of leaf traces in the seedlings of *D. spinulosum*. Thus we find numerous strands (varying from 7 to 9) passing obliquely upward into each leaf base, each one of which causes a gap of its own in the main stele. As previously indicated, however, these strands do not all enter the petiole. There is instead an anastomosis of traces in the sheathing base of the leaf, resulting in the conspicuous and characteristic horizontal girdles, which correspond in many respects to the marginal vein of

the sheathing monocotyl leaves, save that the marginal vein of the typically sheathing monocotyl leaf is connected with a large number of bundles which come off around the entire periphery of the stem.

Summary

1. The medullary rays of *Dioon spinulosum* are of three distinct types: uniseriate rays, a single layer of cells wide and several cells deep; multiseriate rays, two to several cells in width and of variable longitudinal extent; and broad foliar rays or leaf gaps, which, with their included leaf traces, are such a constant feature of this wood.

2. The fibrovascular elements constituting the leaf traces in the foliar rays and connecting these traces with the secondary wood are peculiar, irregular scalariform tracheids which in the course of their development curve gradually downward through the ray, until they become inserted between the perpendicular fibrous elements of the main stele.

3. The regular pitted elements of the secondary xylem are also often diverted to one side into a direction parallel to that of the trace.

4. Both the scalariform and the pitted elements constituting these traces, in their peculiar manner of enlargement and elongation, furnish excellent illustrations of gliding growth.

5. For each leaf or scale leaf 7-9 strands (the number varying with the size of the leaf base) separate from the vascular cylinder. The two inner ones, arising from the proximal side of the central cylinder, pursue a more or less direct vertical course into the ventral part of the petiole; the rest of the traces, leaving the stem cylinder at different points, pass obliquely upward into the cortex and the sheathing base of the leaf, where an anastomosis of traces takes place, resulting in the two characteristic girdles.

6. The two direct strands entering the ventral or abaxial part of the leaf may also unite with the two dorsal girdling strands at the base of the petiole, so that the whole system is reducible in the older seedlings and adult stem to two main horizontal strands with their associated lateral traces.

Grateful acknowledgment is made of the helpful criticism and advice given by Professors JOHN M. COULTER, CHARLES J. CHAMBERLAIN, and W. J. G. LAND during the progress of the investigation. The writer is also greatly indebted to Dr. CHAMBERLAIN for the very generous supply of material.

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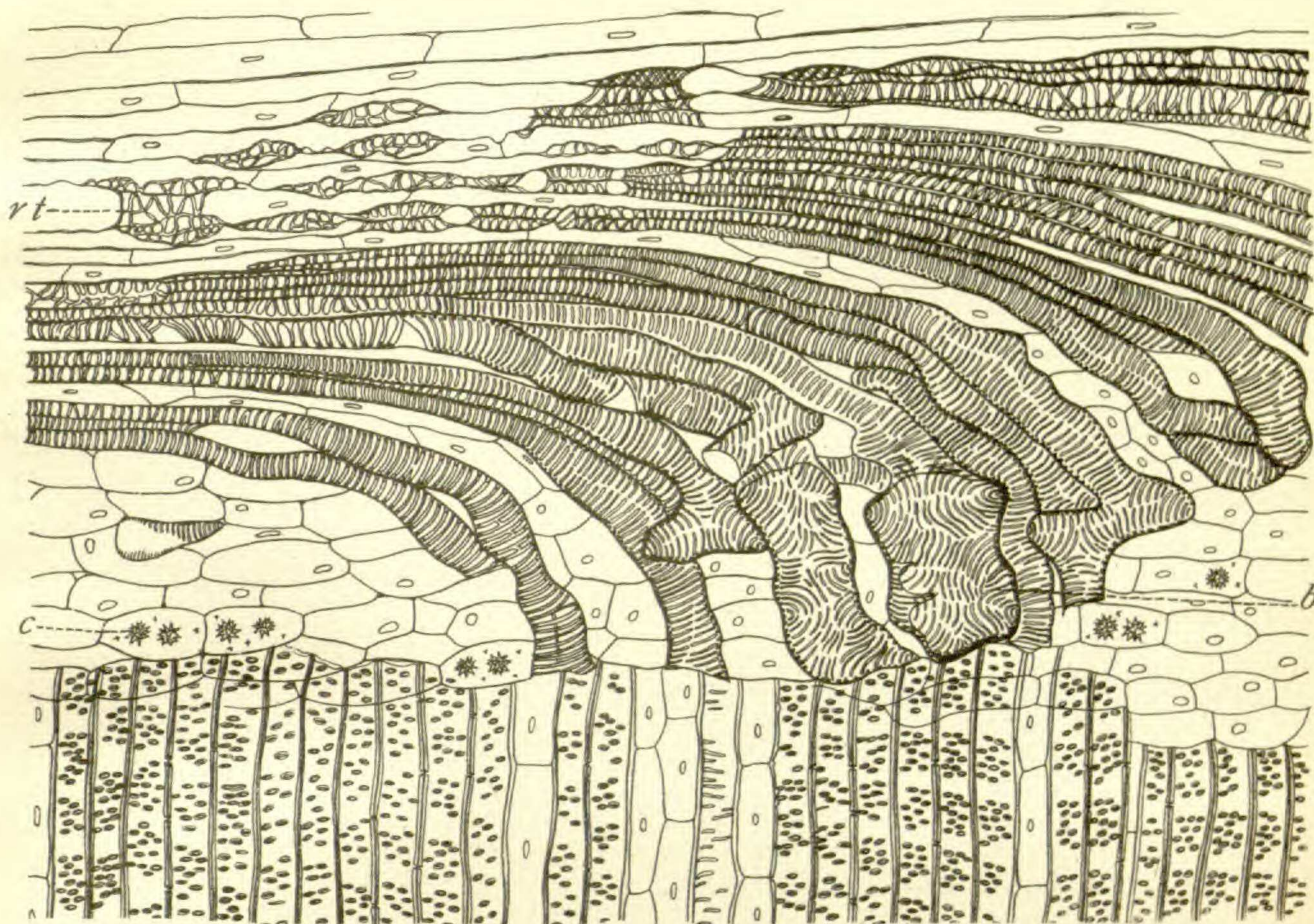
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EXPLANATION OF PLATES XV-XVII

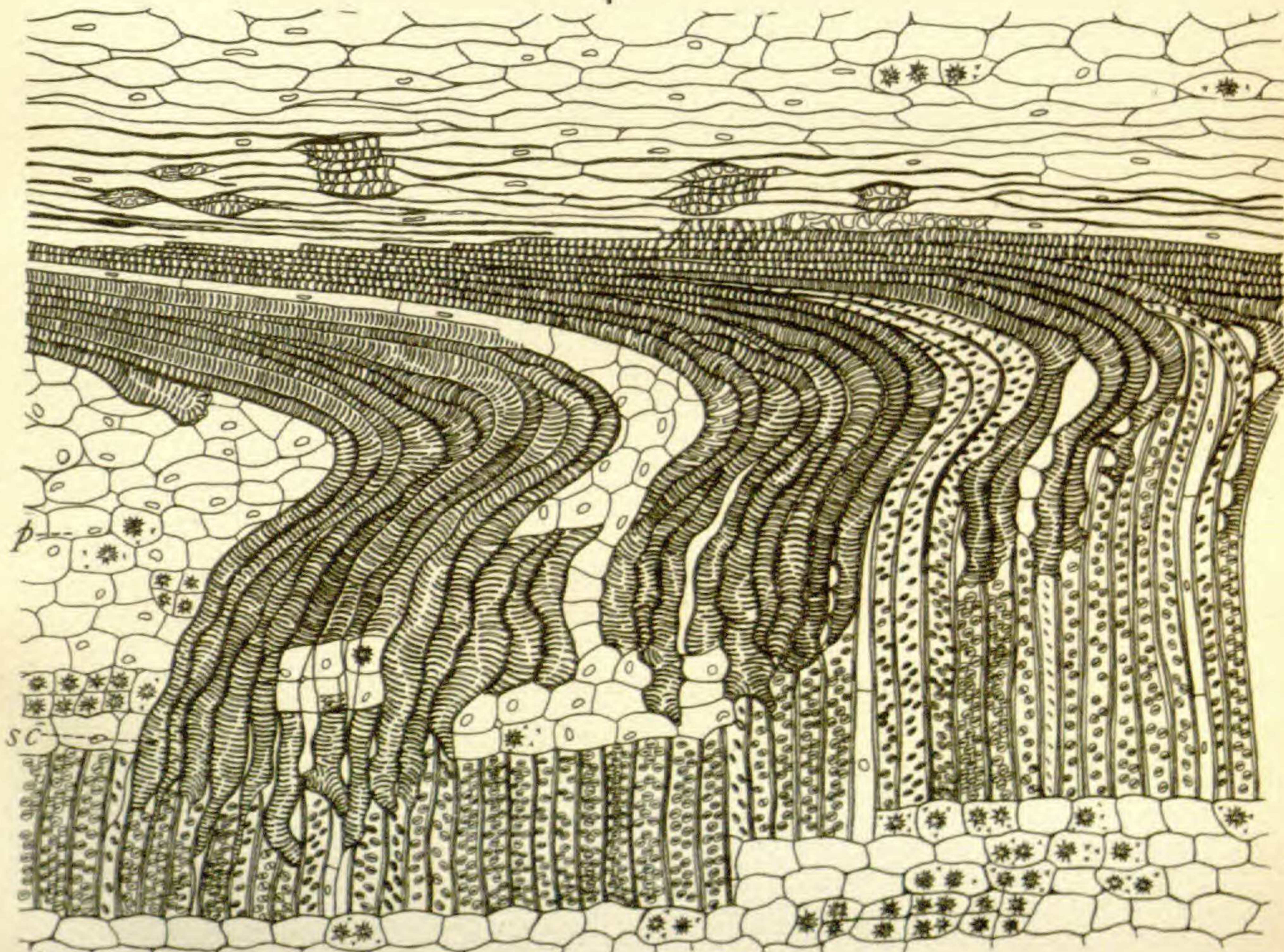
All the drawings were made with the aid of a camera lucida, except figs. 8, 9, 10, 11, which are diagrams showing origin and distribution of foliar vascular strands, as traced from cleared material, supplemented, where detail in connection was required, by serial sections; figs. 1-3 and 12 are in the text.

PLATE XV

FIG. 4.—Mature wood: radial longitudinal section of large foliar ray, showing organization of leaf trace and structure of scalariform tracheids of trace: *b*, bulbous bases of tracheids; *c*, calcium oxalate crystals; $\times 90$.



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